membrane fusion is viral strain independent. Cryo-ET of fusion products between liposomes and isolated VLP containing depalmitoylated HA revealed accumulation of small arrested fusion pores with an average internal diameter of 2 nm.

1776-Plat
Prefusion Structures of Lipid-Bound SNARE Proteins Suggest Folding Pathways of Trans-SNARE Complex
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The assembly of three neuronal soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor (SNARE) proteins synaptobrevin 2, syntaxin-1A, and SNAP-25 is the key step that leads to exocytotic fusion of synaptic vesicles. In the fully assembled SNARE complex, these three proteins form a coiled-coil four-helix bundle structure by interaction of their respective SNARE motifs. Although biochemical and mutational analyses strongly suggest that the heptad-repeat SNARE motifs zipper into the final structure in the N- to C-terminal direction, little is known about the prefusion state of individual membrane-bound SNAREs and whether they change conformation from the unzippered prefusion to the zippered postfusion state in a continuous or step-wise fashion in membrane environments. We have solved the solution NMR structures of micelle-bound synaptobrevin and syntaxin-1A in their prefusion conformations. In addition to their respective transmembrane helices, the SNARE motifs of both proteins have considerable degrees of helical content. For synaptobrevin, only the N-terminal half (residues 36-54) of the SNARE motif forms a transient helix, and the fraction of helical content and interfacial association decreases as the protein is moved from micelle to bicelle to bilayer environments, suggesting that membrane curvature affects the folding of synaptobrevin. For syntaxin, the SNARE motif consists of two well-ordered, membrane-bound helices separated by the "0-layer" residue. These unexpected structural orders of the N- and C-terminal halves of the prefusion SNARE motifs suggest the formation of partially zippered SNARE complex intermediates. Interferometric fluorescence measurements in lipid bilayers confirm that the open SNARE motif helices interact with lipid bilayers and that the assembly of SNARE complexes involves the segmented movements of N- and C-terminal halves of SNARE motifs in relation to the membrane surface.

1777-Plat
Energetics and Kinetics of SNARE Zippering and Regulation Revealed by Single-Molecule Manipulation Approach
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Soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins are evolutionarily conserved molecule machines that couple their folding/assembly to drive diverse intracellular membrane fusion. In particular, neuronal SNAREs mediate extremely fast and calcium-triggered fusion of synaptic vesicles in nerve endings for neurotransmission. It remains unclear how SNAREs fold and how such folding is coupled to membrane fusion and regulated in response to different stimuli. To address these questions, we have characterized the folding energy and kinetics of four representative SNARE complexes at a single-molecule level using high-resolution optical tweezers. Despite their dramatically different fusion rate, all four SNARE complexes assemble by the same step-wise zippering mechanism: slow N-terminal domain (NTD) association, a pause in a force-dependent half-zippered intermediate and fast C-terminal domain (CTD) zippering. However, the energy release from CTD zippering differs from 13 kBT to 28 kBT for yeast and neuron SNARE complexes, respectively. We suggest that SNARE complexes share a conserved zippering pathway to efficiently drive membrane fusion, but release different amount of energy to control the fusion speed. Finally, we will show our latest results about the roles of SNARE mutations, Munc18-1, complexin, and Vc peptides in SNARE zippering and membrane fusion.

1778-Plat
Temporally Resolving Protein and Lipid Colocalization at Exocytic Sites in INS-1 Cells
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The controlled release of material from cells, called exocytosis, is critical for many life processes ranging from synaptic transmission to hormone secretion. Exocytosis is mediated by the assembly of SNARE proteins into a complex that links vesicular and plasma membranes and promotes membrane fusion. Despite a detailed understanding of SNARE complex assembly, a comprehensive description of the arrival and departure of the myriad of other proteins involved in exocytosis is lacking. Here, we describe the localization and dynamics of over a dozen proteins at sites of dense core vesicle exocytosis inside living INS-1 cells. We use two color total internal reflectance fluorescence microscopy to visualize transiently transfected, fluorescently tagged proteins. NPY-GFP, a dense core vesicle cargo protein, is used to localize vesicles and temporally align many exocytic events across several cells. mCherry is used to localize a particular exocytic protein of interest relative to the dense core vesicle and determine its temporal behavior relative to the moment of fusion. Intriguingly, we find that both positive and negative regulators of SNARE complex assembly appear to be present at exocytic sites and diffuse away after fusion occurs. This data suggests that effectors acting on the SNARE complex must be biochemically or conformationally regulated; they are not spatially regulated by exclusion or inclusion at exocytic sites. We are currently examining one exocytic protein, tomosyn, to determine how this protein might be regulated. We are also investigating lipid dynamics at the site of exocytosis using fluorescently tagged lipid binding domains. Our imaging approach should show whether critical lipids are locally synthesized or laterally diffuse to the site of exocytosis. With our data we seek to build a thorough description of protein and lipid behavior at the moment of exocytosis.

1779-Plat
Defining a Retrovirus Entry Site by Single Particle Tracking
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Current knowledge of viral entry pathways and therefore of host factors involved in virus trafficking to the sites of entry is limited. We imaged single Avian Sarcoma and Leukosis Virus (ASLV) co-trafficking with markers for early (Rab5) and late (Rab7) endosomes and visualized the acidification of endosomal lumen and subsequent virus-endosome fusion. The recruitment of Rab5 marker by virus-carrying endosomes usually coincided with acidification of their lumen, which triggered ASLV fusion. Fusion measured by the viral content release occurred either in early (Rab5-positive) or intermediate (Rab5- and Rab7-positive), maturing endosomes. Expression of different isoforms of the cognate ASLV receptor (TVA) on target cells mediated virus entry from distinct compartments. In cells expressing the transmembrane TVA950 receptor, ASLV preferentially entered and fused with slowly maturing early endosomes, which accumulated Rab7 after a considerable delay. Viral entry occurred from either slowly or quickly maturing endosomes in cells expressing the GPI-anchored TVA800 receptor, as manifested by ASLV fusion with early (Rab5-positive) or intermediate (Rab5- and Rab7-positive) compartments. Simultaneous visualization of endosome acidification and viral content release enabled the measurement of the true kinetics of viral fusion with endosomes, which was independent of the receptor isoform. We concluded that the sites of ASLV entry are determined by the kinetic competition between endosome maturation and low pH-dependent fusion. These findings demonstrate the ability of ASLV to enter cells *via* alternative endocytic pathways and establish infection by fusing with distinct endosomal compartments. This work was partially supported by the NIH R01 AI053668 grant.